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Early ultrastructural changes in the cerebral cortex of albino rats subjected to 3-aminopyridine seizures

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Summary. The ultrastructural effects of the convulsant 3-aminopyridine were studied. It was found that astrocytic processes suffered swelling, while pyramidal cells underwent shrinkage and condensation in the 2nd and 3rd layers of rat cerebral cortex.

Recent electrophysiological investigations demonstrated that 3-aminopyridine (3-AP) applied locally on the cerebral cortex of adult cats caused convulsive phenomena within 5 min[2]. The present study concerning the ultrastructure of the 3-AP seizure focus, has been initiated because clear-cut morphological changes indicating facilitated transmitter release were found at spinal cord synapses after i.v. 4-aminopyridine administration[3,4]. To our knowledge, no electron microscopic studies have analyzed so far the morphological changes in neocortical aminopyridine seizures.

Materials and methods. Experiments were carried out on CFY strain male and female albino rats weighing 300-450 g. The fronto-parietal border region of the neocortex[5] was exposed on 1 side under chloralose-urethane anesthesia. We placed Gelaspon (VEB Jenapharm) soaked in 37 °C 0.9% NaCl on the meningeal surface and put a 2 mg 3-AP crystal (Koch-Light Labs Ltd) on the wet Gelaspon. Spontaneous jerks of the head and neck musculature occurred 2-4 min after the 3-AP application. Control animals were operated in the same manner except for the application of 3-AP. 3-AP treated animals were perfused 10 min after the beginning of the manifest muscle jerks, control animals 10 min after the Gelaspon application, both with a formaldehyde-glutaraldehyde fixative solution[6] for 20 min. Animals were decapitated after perfusion and their heads immersed in the same fixative overnight. After fixation small pieces were excised from the cerebral cortex, osmicated and embedded in araldite (Fluka). Ultrathin sections stained with lead citrate[6] were examined with a Tesla BS 500 electron microscope. Ultrastructural changes found in the cortical tissue treated with 3-AP (referred to as the primary focus) were compared to the control operated side.

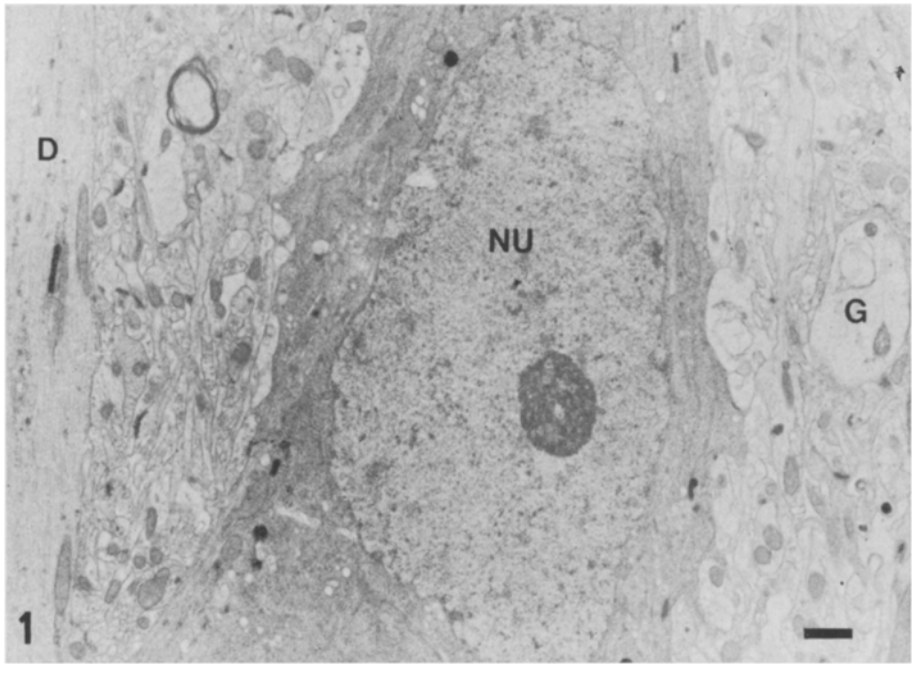

Fig. 1. Shrunken pyramidal cell in the 3rd layer of the cerebral cortex in the primary focus. Note the increased electron density of the neuronal cytoplasm and the swollen glial process at G. D, dendrite; NU, pyramidal cell nucleus. Bar represents 1.0 µm.

Results. The detailed electron microscopic analysis of the cerebral cortex from control operated animals showed normal ultrastructure[7,8], but in the 1st layer there was always remarkable swelling of astrocytic processes. In the primary focus, however, the astrocytic swelling (figs. 1-3) extended from layer 1 to layer 5. In the neurons of the 2nd and 3rd layers slight enlargement of Golgi vacuoles, swelling of mitochondria and a lot of free cytoplasmic ribosomes were seen. In addition, some pyramidal cells in these layers underwent considerable shrinkage and condensation (fig. 1), and thin cytoplasmic leaflets extending from their soma into the neuropil could be seen (fig. 2). The dendrites of these pyramidal cells were also shrunken, their electron density was considerably higher than that of the other neurons in these layers and of the pyramidal cells from control animals. We observed enlarged presynaptic axon terminals containing swollen mitochondria and terminating on dilated or shrunken dendritic segments. Some axon terminals presynaptic to the dendrites of the 2nd and 3rd layers were almost empty: there were only 2-3 vesicles attached to the presynaptic membrane, in the terminal axoplasm.

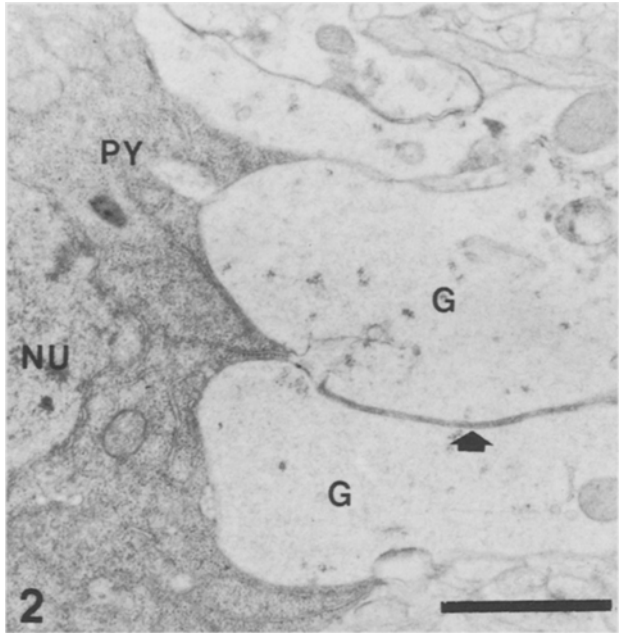

Fig. 2. Swollen astrocytic processes (G) and the cytoplasmic leaflets (arrow) of a shrunken pyramidal cell body in the 2nd layer of the cerebral cortex (PY, the perinuclear cytoplasm of the shrunken pyramidal cell; NU, cell nucleus). Bar represents 1.0 µm.

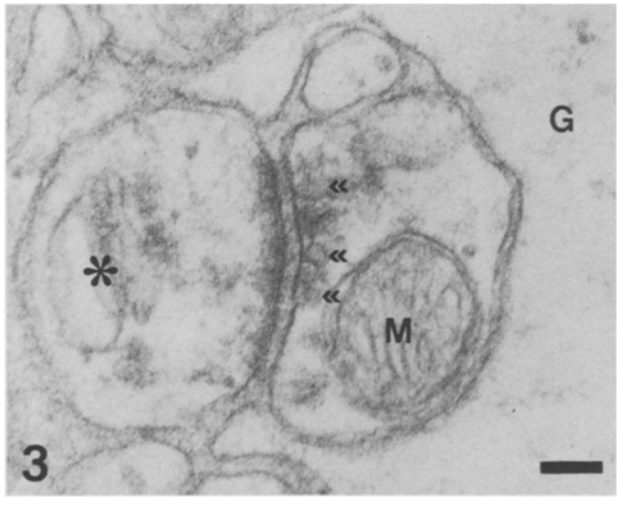

Fig. 3. Axo-spinodendritic synapse from the 3rd layer of the cerebral cortex in the primary focus. Note the paucity of synaptic vesicles (double arrowheads) and the swollen glial process at G (asterisk, spine apparatus; M, mitochondrion). Bar represents 0.1 µm.

Discussion. What we have found in the primary focus, i.e. the swelling of astrocytes, was a frequent finding in acute experimental seizures[9-12]. While the edematous changes have been reported to be of pathogenic importance in seizures developed in experimental allergic encephalitis[13] there seemed to be no reason to suppose a similar role of edema in the precipitation of aminopyridine convulsions. The swelling of presynaptic axon terminals and the depletion of their vesicle content fit in much better with the electrophysiological findings on the specific presynaptic action of aminopyridines[14], while the shrunken postsynaptic structures and pyramidal cell bodies may reflect the enormous level of neuronal excitation[15] as a consequence of the primary 3-AP action at the presynaptic terminals. The concomitant shift of electrolytes and water from neuronal processes to astrocytes probably alters extracellular ion concentrations and thus contributes to the maintenance of neuronal excitation[16] and manifests itself as an extensive glial swelling. Because of the immediate development of these ultrastructural changes, the 3-AP focus seems to be a valuable object in the study of the early synaptic phenomena[17] accompanying neocortical convulsions.

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